

CLAIMS

1. An isolated polynucleotide derived from a fungal source, which polynucleotide comprises a nucleotide sequence encoding an enzyme having  $\beta$ -glucosidase activity. ✓
2. An isolated polynucleotide selected from the group consisting of: ✓
  - (a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL6 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL6 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL6 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
  - (d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL6 polypeptide having the amino acid sequence presented in Figure 2;
  - (e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL6 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
  - (f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL6 polypeptide having the amino acid sequence presented as SEQ ID NO:2;
  - (g) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof; and
  - (h) a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a  $\beta$ -glucosidase
3. The isolated polynucleotide of Claim 2, wherein % identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

4. The isolated polynucleotide of Claim 2, wherein hybridization is conducted at 42°C in 50% formamide, 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured carrier DNA followed by washing two times in 2X SSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42°C.

5. The isolated polynucleotide of Claim 2, wherein said polynucleotide is an RNA molecule.

6. The isolated polynucleotide encoding an enzyme having β-glucosidase activity, wherein the enzyme is derived from a *Trichoderma* source. ✓

7. The isolated polynucleotide of Claim 6, wherein the enzyme is derived from *Trichoderma reesei*.

8. An expression construct including a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2), or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence disclosed in Figure 2 under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2). ✓

9. A vector including the expression construct of Claim 8.

10. A vector comprising an isolated polynucleotide of Claim 2, operably linked to control sequences recognized by a host cell transformed with the vector.

11. A host cell transformed with the vector of Claim 9.

12. A host cell transformed with the vector of Claim 10.

13. The host cell of Claim 12, which is a prokaryotic cell.

14. The host cell of Claim 12, which is a eukaryotic cell.

15. A recombinant host cell comprising a polynucleotide of Claim 2.

16. The recombinant host cell of Claim 15, which is a prokaryotic cell.
17. The recombinant host cell of Claim 15, which is a eukaryotic cell.
18. A substantially purified BGL6 polypeptide with the biological activity of a  $\beta$ -glucosidase, comprising a sequence selected from the group consisting of:
  - (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
  - (d) an amino acid sequence presented in Figure 2;
  - (e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
  - (f) an amino acid sequence presented as SEQ ID NO:2;
  - (g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.
19. A method of producing an enzyme having  $\beta$ -glucosidase activity, comprising:
  - (a) stably transforming a host cell with an expression vector comprising a polynucleotide as defined in Claim 2;
  - (b) cultivating said transformed host cell under condition suitable for said host cell to produce said  $\beta$ -glucosidase; and
  - (c) recovering said  $\beta$ -glucosidase.
20. The method of Claim 19 wherein the host cell is a filamentous fungi or yeast cell.
21. A purified enzyme having  $\beta$ -glucosidase activity prepared by the method of Claim 19.
22. A recombinant host cell comprising a deletion or insertion or other alteration in the *bgl6* gene which inactivates the gene and prevents BGL6 polypeptide production.
23. An antisense oligonucleotide complementary to a messenger RNA that encodes a BGL6 polypeptide having the sequence presented as SEQ ID NO:2, wherein

upon exposure to a  $\beta$ -glucosidase-producing host cell, said oligonucleotide decreases or inhibits the production of  $\beta$ -glucosidase by said host cell.

24. The antisense oligonucleotide of Claim 23, wherein the host cell is a filamentous fungi.

25. A detergent composition, said composition comprising a polypeptide selected from the group consisting of:

- (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
- (d) an amino acid sequence presented in Figure 2;
- (e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
- (f) an amino acid sequence presented as SEQ ID NO:2;
- (g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.

26. A method of improving the characteristics of a yeast dough or baked good made from such dough consisting essentially of the steps:

- (a) mixing at least about 10 ppm of a BGL6 according to Claim 18 with dough ingredients to form a dough mixture, and
- (b) baking said dough mixture to form a baked good.

27. A method of improving yeast bread dough or yeast roll dough or yeast bread or yeast roll characteristics consisting essentially of the steps:

- (a) mixing at least about 10 ppm of a BGL6 according to Claim 18 with bread or roll dough ingredients to form a dough mixture;
- (b) shaping or panning the dough mixture;
- (c) proofing the dough mixture, and
- (d) baking the dough mixture to form bread or rolls.

28. A method of expressing a heterologous polypeptide having  $\beta$ -glucosidase activity in an *Aspergillus* species, comprising:

(a) Providing a host *Aspergillus* with an expression vector comprising a polynucleotide encoding an *Aspergillus*  $\beta$ -glucosidase signal sequence linked to a polynucleotide encoding a heterologous  $\beta$ -glucosidase, thereby encoding a chimeric polypeptide;

(b) Cultivating said host *Aspergillus* under conditions suitable for said *Aspergillus* to produce said chimeric polypeptide, wherein said chimeric polypeptide is produced.

29. A method of producing ethanol, said method comprising the steps of:

- a) contacting a biomass composition with an enzymatic composition comprising  $\beta$ -glucosidase 4 to yield a sugar solution;
- b) adding to the sugar solution a fermentative microorganism; and
- c) culturing the fermentative microorganism under conditions sufficient to produce ethanol,

wherein the biomass composition may be optionally pretreated.

30. The method of claim 29 wherein step (a) further comprises the addition of at least one endoglucanase.

31. The method of claim 29 wherein step (a) further comprises the addition of at least one cellobiohydrolase.

32. The method of claim 30 wherein step (a) further comprises the addition of at least one cellobiohydrolase.

33. The method of claim 29 wherein the pretreatment is with a dilute acid.

34. A method of producing ethanol, said method comprising the steps of:

- a) contacting a biomass composition with an enzymatic composition comprising a  $\beta$ -glucosidase and a fermentative microorganism; and
- b) culturing the fermentative microorganism under conditions sufficient to produce ethanol,

wherein the biomass composition may be optionally pretreated.

35. The method of claim 34 wherein step (a) further comprises the addition of at least one endoglucanase.

36. The method of claim 34 wherein step (a) further comprises the addition of at least one cellbiohydrolase.

37. The method of claim 35 wherein step (a) further comprises the addition of at least one cellbiohydrolase.

38. The method of claim 34 wherein the pretreatment is with a dilute acid.